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Multiple Transcription-Activating Sequences Regulate the RsmZ Regulatory Small RNA of *Pseudomonas brassicacearum*

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The *mutS-rpoS* region is known to be a highly polymorphic segment of the chromosome owing to horizontal gene transfer and evolutionary processes. In *Pseudomonas*, *mutS-fdxA-rsmZ-rpoS* organization is highly conserved, as well as the promoter region of the RsmZ small RNA (sRNA)-encoding gene. One exception to this conservation is in *Pseudomonas brassicacearum*, where a 308-nucleotide (nt) sequence, predicted to form a hairpin structure in single-stranded DNA (ssDNA), is inserted between the *rpoS* and *rsmZ* genes. Using MEME software, we identified nine consensus motifs in the *rsmZ* promoter region of 16 sequenced *Pseudomonas* genomes. We observed that an upstream activation sequence (UAS) and an M1 motif (located between the −10 promoter element and the UAS) are shared among examined *Pseudomonas* genomes. A third motif, the M2 motif, is localized within the coding sequence of the *rpoS* gene. Constructs fusing the different identified motifs to the *lacZ* reporter were produced. Our *in vivo* analysis of the *rsmZ*-activating elements indicates that the palindromic UAS located 180 bp upstream of the *rsmZ* transcriptional start in *P. brassicacearum* NFM 421 is essential, but not sufficient, for full *rsmZ* expression. Here, we demonstrate a role for the three motifs in the activation of the *rsmZ* gene, and we hypothesize the role of additional transcriptional factors, along with the DNA structuring role of the hairpin in the complex network controlling the expression of *rsmZ*.

Bacteria sense the overall environmental stimuli and adjust their diverse physiological processes to ensure a coordinated and effective survival response. Regulatory small RNAs (sRNAs) are key players that adapt metabolism in response to rapid environmental changes. They are known to participate in several bacterial regulatory networks and to modulate a wide range of physiological responses in bacteria (28).

In *Pseudomonas*, Rsm (regulator of secondary metabolism) sRNAs act as decoys to inhibit the activity of posttranscriptional regulatory proteins (RsmA/RsmE), which are in turn repressors of secondary metabolite synthesis. The GacS/GacA two-component system is a signal transduction pathway that activates the transcription of these sRNA-encoding genes (19).

In the beneficial plant root-associated bacterium *Pseudomonas brassicacearum*, transcription of three sRNA-encoding genes (*rsmX*, *rsmY*, and *rsmZ*) is positively activated by the GacS/GacA system. These sRNAs act redundantly to control traits required for phytoprotection from disease (i.e., antimicrobial compounds and lytic enzymes) and for phytostimulation (i.e., auxin production) (18).

In *P. brassicacearum* (1, 24), the *rsmZ* gene, which exhibits a high level of expression, is likely not exclusively activated by the GacS/GacA system (18). In *Pseudomonas*, the genetic context of the *rsmZ* gene is well conserved (4, 12, 15); *rsmZ* is located between the *mutS* and *rpoS* genes. MutS is part of the mismatch repair (MMR) system, and RpoS is an alternative sigma factor. Interestingly, in gammaproteobacteria the *mutS* and *rpoS* genes are located in a highly polymorphic segment of the chromosome. The *mutS-rpoS* intergenic region of enteric bacteria ranges in size from 88 bp in *Yersinia enterocolitica* to >12,000 bp in *Salmonella* (16). Even within the shortest *mutS-rpoS* intergenic region, size variation is observed among strains of the same species. The heterogeneity of the *mutS-rpoS* regions reflects the environmental pressure exerted on this genomic region. Thus, local sequence variation suggests that horizontal gene transfer and evolutionary processes have occurred in the *mutS-rpoS* intergenic region (9, 23).

In this study, we demonstrate that whereas the *mutS-rpoS* region is highly polymorphic in enterobacteria, it is quite homogenous in *Pseudomonas*, where the genetic context *mutS-fdxA-rsmZ-rpoS* appears to be almost stabilized.

We also present several experiments that collectively support a claim for additional control elements for *rsmZ* in the coding sequence of *rpoS* and in the *rpoS-rsmZ* intergenic region in the *P. brassicacearum* strain NFM 421.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table S1 in the supplemental material. *Pseudomonas brassicacearum* NFM 421 and the Δ*gacA* mutant were grown in Trypticase soy broth 10-fold diluted (TSB/10) (BD) at 30°C.

For growth on plates, medium was solidified with 15 g/liter agar (Sigma).

DNA and RNA manipulation. Plasmids were extracted with a QIAprep Spin Miniprep Kit (Qiagen), and DNA fragments were purified from agarose gels with a QIAquick Gel extraction Kit (Qiagen) according to the manufacturer's instructions.

Construction of transcriptional *lacZ* fusions. To construct transcriptional *lacZ* fusions, promoter regions were amplified by PCR with primers listed in Table S1 in the supplemental material. The PCR products were

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subsequently digested with specific restriction enzymes and cloned into pME6016 plasmids (27) and then sequenced.

β-Galactosidase assays. Strains containing sequence motif-*lacZ* fusion constructs were grown overnight, diluted 1:200 in 8 ml of TSB/10 with tetracycline (20 μg/ml), and grown over a period of 24 h. β-Galactosidase activities were quantified as previously described (18).

***mutS-rpoS* sequence analysis.** Polymorphic *mutS-rpoS* regions from 80 sequenced genomes are reported in Table 1. Results were obtained from 758 sequenced genomes.

***rsmZ* promoter motif search.** Using MEME software (Multiple Em for Motif Elicitation [2]) with the "zoops" setting (zero or one occurrence per sequence), we searched for up to 15 conserved sequence motifs in the *rsmZ* promoter region of 16 sequenced *Pseudomonas* genomes (*Pseudomonas putida* KT2440, *P. putida* F1, *P. brassicacearum* NF421, *Pseudomonas syringae* pv. phaseolicola 1448A, *P. syringae* pv. syringae B728a, *P. syringae* pv. tomato DC3000, *Pseudomonas fluorescens* F113, *P. fluorescens* Pf0-1, *P. fluorescens* Pf-5, *P. fluorescens* SBW25, *Pseudomonas entomophila* L48, *Pseudomonas aeruginosa* PA7, *P. aeruginosa* PAO1, *P. aeruginosa* LESB58, *Pseudomonas mendocina* ymp, and *Pseudomonas stutzeri* A1501). The default parameters were used for all other MEME settings. Nine motifs were identified (see Table S2 in the supplemental material).

RESULTS AND DISCUSSION

The polymorphic *mutS-rpoS* region and acquisition of genes. The polymorphic *mutS-rpoS* region of 80 sequenced genomes is reported in Table 1. This region is mainly conserved in gammaproteobacteria but may be extended to some deltaproteobacteria such as *Geobacter* and cyanobacteria such as *Synechococcus*. The distance between *mutS* and *rpoS* varies from 40 bp in *Idiomarina loihiensis*, a deep-sea-living bacteria, to more than 13,000 bp in some *Salmonella* strains and can include from 0 to 14 inserted genes.

Polymorphism is mainly observed in *Enterobacteriaceae* including *Escherichia coli*, *Salmonella*, *Shigella*, and *Citrobacter koseri*. These bacterial species inhabit mammalian intestines or other tissue. The *mutS-rpoS* region was likely used during evolution to integrate acquired genetic determinants that allow the bacteria to respond to the host niche (13, 23). Indeed, genes acquired by horizontal transfer and inserted in the *mutS-rpoS* region are involved in the adaptation of bacteria to their environments (i.e., antibiotic resistance, metabolic properties, chemotaxis, virulence, etc.).

***mutS-rpoS* region in *Pseudomonas*.** In *Pseudomonas*, the *mutS-rpoS* region appears to be almost stabilized (Fig. 1). That is, organization of *mutS-fdxA-rsmZ-rpoS* is highly conserved within *Pseudomonas*, with the exception of *Pseudomonas fluorescens* SBW25 and *Pseudomonas syringae* pv. tomato DC3000 (in which the *mutS* and *rpoS* genes are distant from each other, at 137,082 bp and 2,835,459 bp, respectively) and of *P. fluorescens* F113 and *Pseudomonas putida* KT2440, where an additional transposase or group II intron-encoding maturase is inserted (Table 1). The latter is a unique class of catalytic RNAs (ribozymes) that is capable of self-splicing and may encode a multifunctional protein (intron-encoded protein [IEP]) that has reverse transcriptase (RT) activity (6). Apart from these exceptions, the distance between *mutS* and *rpoS* is relatively constant. It ranges from 816 bp for *P. aeruginosa* PA7 to 1,365 bp for *P. brassicacearum* because of the insertion of 308 bp between the *rpoS* and *rsmZ* genes (Fig. 1). This inserted sequence is predicted to form a hairpin structure in single-stranded DNA (ssDNA).

The gene *fdxA* encodes a ferredoxin involved in electron transfer, and the gene *rsmZ* encodes a regulatory small RNA (sRNA). RsmZ is part of Rsm regulatory system, which is homologous to the Csr system (carbon storage regulator) in *E. coli* (19). Functional homologs of the Csr/Rsm system have been discovered in many Gram-negative bacteria, including *Escherichia* (21), *Salmonella* (11), *Vibrio* (20), *Yersinia* (14), *Azotobacter* (7), *Acinetobacter* (8), *Erwinia* (22), *Serratia* (29), and *Photorhabdus* (17). Although these bacteria possess a functional Csr/Rsm system, Csr/Rsm sRNAs are not systematically located between *mutS* and *rpoS* (Table 1), which is a unique feature in gammaproteobacteria.

Regulation of the *rsmZ* gene. The GacS/GacA two-component system in *Pseudomonas* activates *rsm* expression (19) via an upstream activating sequence (UAS) in the promoter region (Fig. 2). More precisely, in *P. aeruginosa* and *P. fluorescens*, the UAS is necessary for *rsmZ* expression but is not sufficient, and additional regulators may be involved (4, 15). This suggests that the *rsmZ* promoter region could be more complex than in other *rsm* genes. Indeed, in all studied *Pseudomonas* species, the UAS is located 188 bp upstream of the +1 transcriptional start of *rsmZ*, whereas it is almost 70 bp upstream of the other *rsm* genes (4, 15, 18).

In *P. brassicacearum*, *rsmZ* is not regulated exclusively by GacA (18). Using MEME software, we searched for conserved motifs in the *rsmZ* promoter regions of 16 sequenced *Pseudomonas* genomes. Two potential regulatory elements were found in all 16 *Pseudomonas* species, including *P. fluorescens* F113, *P. putida* KT2440, *P. syringae* pv. tomato DC3000, and *P. fluorescens* SBW25, which do not share the same *mutS-rpoS* organization. The first regulatory element corresponds to the previously identified UAS, required for GacA control (19), while the second one (M1 motif) corresponds to a region localized between the transcriptional start of *rsmZ* and the UAS. In addition, six motifs (M2 to M7) were found that are within *rpoS*, near the 3′ end of the coding sequence (see Table S2 in the supplemental material). These motifs could be regulatory elements or could reflect conservation of the RpoS protein sequence.

To test whether the M1 motif or the sequences within *rpoS* (M2 to M7) are required for the regulation of *rsmZ* in *P. brassicacearum*, we produced different constructs fused to the *lacZ* reporter, and we tested their expression in the wild type and in the *gacA* mutant (Fig. 2).

The UAS motif is essential for *rsmZ* expression. Deletion of the UAS motif in the *rsmZ*-ΔUAS-*lacZ* fusion (Fig. 2) shows a drastic decrease in the *rsmZ* gene expression level even if the other potential activating sequences (M1 and M2 motifs) are present. Data demonstrate that the UAS motif is essential for *rsmZ* gene expression as in other *Pseudomonas* species (4, 15).

M1 motif, localized between the transcriptional start and the UAS. In *P. brassicacearum*, the A/T-rich M1 motif (Fig. 3A) is localized −117 to −78 bp upstream of the transcriptional start of *rsmZ*. Analysis of *rsmZ* expression using an *rsmZ*-M1-*lacZ* fusion (which includes a fragment that extends from M1 motif to the +1 start of *rsmZ*) reveals *rsmZ* gene activation in the absence of the UAS. When the M1 sequence is deleted in an *rsmZ*-NM-*lacZ* fusion (where NM stands for no motif) or in a *rsmZ*-ΔM1-*lacZ* fusion, a loss of expression is observed, indicating that the M1 motif is a recognition site for one or more regulatory factors. Additionally, expression analysis with the *rsmZ*-M1-*lacZ* fusion showed a decrease in the *gacA* mutant in comparison to the wild-type level (Fig. 2), indicating that activation of *rsmZ* expression from the M1 motif is at least partially GacA dependent. Consequently, the M1 motif is important for the full expression of *rsmZ*.

TABLE 1 Genomic organization of the *mutS-rpoS* region in 80 sequenced genomes

Strain	Size of the *rpoS-mutS* region: Length (bp)	Size of the *rpoS-mutS* region: No. of genes	Organization of the *mutS-rpoS* region[a]: MutS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	RpoS
Azotobacter vinelandii DJ	2,950	3																
Citrobacter koseri ATCC BAA-895	3,068	5																
Enterobacter sp. strain 638	89	0																
Erwinia tasmaniensis	271	0																
Escherichia coli 536	8,953	10																
Escherichia coli APEC O1	8,952	9																
Escherichia coli ATCC 8739	7,083	7																
Escherichia coli CFT073	8,952	11																
Escherichia coli E24377A	9,794	11																
Escherichia coli HS	6,906	7																
Escherichia coli K-12 substrain DH10B	6,905	6																
Escherichia coli K-12 substrain MG1655	6,905	7																
Escherichia coli O157:H7 EDL933	3,744	5																
Escherichia coli O157:H7 Sakai	3,744	5																
Escherichia coli SMS-3-5	3,745	5																
Escherichia coli UTI89	9,531	12																
Geobacter metallireducens GS-15	1,145	3																
Idiomarina loihiensis L2TR	40	0																
Photobacterium profundum SS9	131	0																
Photorhabdus luminescens subsp. *laumondii* TTO1	3,286	2																
Pseudoalteromonas haloplanktis TAC125	73	0																
Pseudomonas aeruginosa PA7	816	1																
Pseudomonas aeruginosa PAO1	817	1																
Pseudomonas aeruginosa UCBPP-PA14	816	1																
Pseudomonas brassicacearum NFM421	1,365	1																
Pseudomonas entomophila L48	936	1																
Pseudomonas fluorescens F113	2,342	2																
Pseudomonas fluorescens Pf-5	980	1																
Pseudomonas fluorescens Pf0-1	977	1																
Pseudomonas putida F1	1,000	1																
Pseudomonas putida GB-1	999	1																
Pseudomonas putida KT2440	2,917	2																
Pseudomonas putida W619	968	1																
Pseudomonas syringae pv. phaseolicola 1448A	1,100	1																
Pseudomonas syringae pv. syringae B728a	1,124	1																
Saccharophagus degradans 2–40	2,506	2																
Salmonella enterica serovar Arizonae 62:z4	6,673	7																
Salmonella enterica serovar Choleraesuis SC-B67	13,956	14																
Salmonella enterica serovar Paratyphi A ATCC 9150	12,598	13																
Salmonella enterica serovar Typhimurium LT2	12,648	14																
Serratia proteamaculans 568	81	0																
Shewanella amazonensis SB2B	116	0																
Shewanella baltica OS155	219	0																
Shewanella baltica OS185	204	0																
Shewanella baltica OS195	204	0																
Shewanella denitrificans OS217	2,689	2																
Shewanella frigidimarina NCIMB 400	385	0																
Shewanella halifaxensis HAW-EB4	120	0																
Shewanella loihica PV-4	260	0																
Shewanella oneidensis MR-1	83	0																
Shewanella pealeana ATCC 700345	113	0																
Shewanella putrefaciens CN-32	97	0																
Shewanella sediminis HAW-EB3	233	0																
Shewanella sp. strain ANA-3	115	0																
Shewanella sp. strain MR-4	141	0																
Shewanella sp. strain MR-7	127	0																
Shewanella sp. strain W3-18-1	97	0																
Shewanella woodyi ATCC 51908	211	0																
Shigella boydii Sb227	6,906	7																
Shigella dysenteriae Sd197	3,798	6																
Shigella flexneri 2a strain 301	9,916	9																
Shigella flexneri 5 strain 8401	10,524	11																
Shigella sonnei Ss046	7,683	7																
Synechococcus sp. strain JA-2-3B a(2-13)	1,176	1																
Synechococcus sp. strain JA-3-3Ab	1,125	1																
Vibrio cholerae O1 biovar El Tor N16961	93	0																
Vibrio cholerae O395	93	0																
Vibrio harveyi ATCC BAA-1116	1,525	1																
Vibrio parahaemolyticus RIMD 2210633	129	0																
Vibrio vulnificus CMCP6	76	0																
Vibrio vulnificus YJ016	76	0																
Yersinia enterocolitica subsp. *enterocolitica* 8081	89	0																
Yersinia pestis Angola	320	1																
Yersinia pestis Antiqua	352	0																
Yersinia pestis biovar microtus 91001	360	0																
Yersinia pestis CO92	328	0																
Yersinia pestis KIM	360	0																
Yersinia pestis Nepa1516	352	0																
Yersinia pestis Pestoides F	328	0																
Yersinia pseudotuberculosis IP 31758	373	0																
Yersinia pseudotuberculosis IP 32953	1,760	1																

(Continued on following page)

TABLE 1 (Continued)

Strain	Size of the *rpoS-mutS* region		Organization of the *mutS-rpoS* region[a]															
	Length (bp)	No. of genes	MutS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	RpoS

[a] Proteins encoded in the order of occurrence according to the following legend:

adenine phosphoribosyltransferase	dehydrogenase	hypothetical protein	putative flavoprotein
aerotaxis sensor	DNA recombination protein	IS/transposase	sigma factor RpoS
aldolase	epimerase	MutS	transcriptional regulator
antibiotic resistance	ferrodoxin, 4Fe-4S	oxidoreductase	transporter/permease
chemotaxis sensory transducer protein	group II intron-encoding maturase	phosphatase	tRNA synthase
decarboxylase	hydroxypyruvate isomerase	phosphoglucomutase	type III effector

Altogether these data suggest that the UAS (and, by extension, GacA) activation cannot account for the full expression of *rsmZ*, suggesting the need for additional regulators.

Activators use different mechanisms of action (5). Due to localization of the M1 motif, we can hypothesize that the regulator(s) involved is a class I activator(s), which binds to a target located upstream of the promoter −35 element and recruits RNA polymerase to the promoter by directly interacting with the RNA polymerase (5).

We can also hypothesize that this A/T-rich motif may be recognized by nucleotide-associated proteins that are known to induce DNA bending, like integration host factor (IHF). IHF has been called the "master bender" as it bends DNA by 150 to 180° (10). Humair et al. (15) found two potential IHF binding sites, located at about −100 and −40 relative to the *rsmZ* transcription start site, while in *P. brassicacearum* one IHF binding site was localized within the M1 motif.

A shared extension of the UAS. The predicted UAS motif appears to be longer than 18 bp. Indeed, an extension of the UAS is likely to be conserved between *P. brassicacearum* and *P. fluorescens*. In *P. brassicacearum*, the extended UAS is **TGTAAGCCTT TGCCTAGT**GTTACGTAAG, whereas **TGTAAGNNNNNNCT TACA** is the more commonly described consensus (boldface indicates the UAS without extension) (19).

When the 10 additional base pairs of the extension [consensus (G/T)T(A/T)$_2$CGTA(A/C)G] were deleted (ΔExt) in an *rsmZ*-ΔExt-*lacZ* fusion, we observed a significant decrease in *rsmZ* expression (Fig. 2) in comparison to the level in the *rsmZ*-M2-*lacZ* fusion (48,787 to 20,220 Miller units).

This extension may be either part of the UAS or a recognition site for a supplementary regulator that potentially interacts with GacA. In *P. fluorescens* CHA0, flanking UASs have been described as being involved in *rsmZ* gene expression (15). For instance, the recognition site of the regulator PsrA, involved in activation of *rsmZ* gene expression, overlaps the UAS and flanking downstream region in *P. fluorescens* CHA0 and *P. fluorescens* Pf-5 (15). In *P. brassicacearum*, we did not observe an influence of *psrA* overexpression on *rsmZ* gene expression (data not shown).

Role of the *rpoS* coding sequence. In *Pseudomonas aeruginosa* PAO1, β-galactosidase activity resulting from a fragment of the *rsmZ* promoter fused to the *lacZ* gene indicates that regions far from the UAS (between −500 and −250 bp upstream of the start of transcription) are essential to the full expression of *rsmZ* (4). Indeed, the presumed site(s) of activation is localized within the *rpoS* coding sequence and enables a 2-fold activation of *rsmZ* expression compared to a shorter *lacZ* fusion. In *P. brassicacearum*, we have predicted six motifs in this region (see Table S2 in the supplemental material). Expression analysis indicates that none of the five upstream motifs (M3 through M7) is involved in the activation of the *rsmZ* gene (data not shown). Furthermore, an *rsmZ*-M2-*lacZ* fusion (with a promoter fragment extending from the M2 motif to the +1 start of *rsmZ*) allowed full expression of the *rsmZ* gene, as shown by comparison to *rsmZ*-M7-*lacZ* and *rsmZ*-IG-*lacZ* fusions (where IG stands for intergenic) (Fig. 2). As

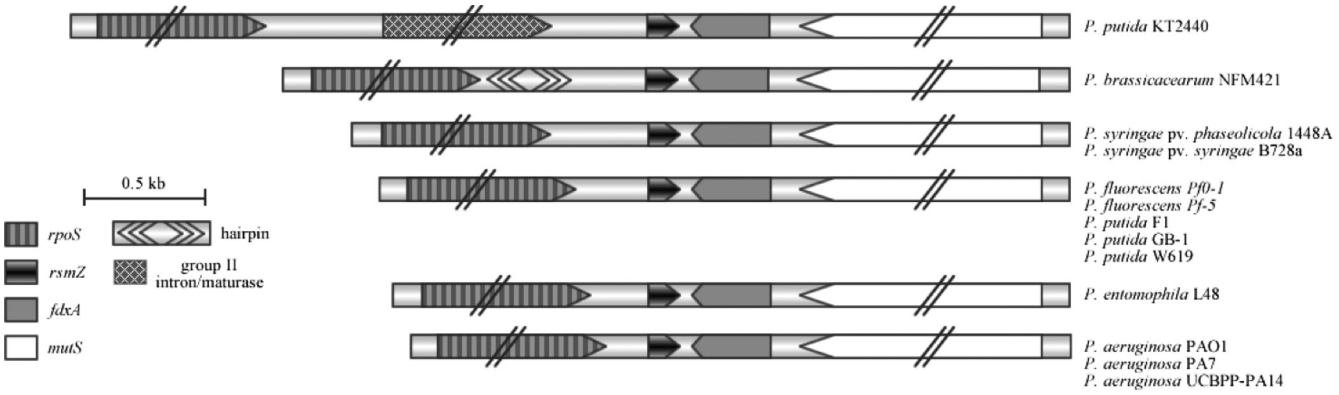

FIG 1 Genomic organization of the *mutS-rpoS* region in *Pseudomonas*.

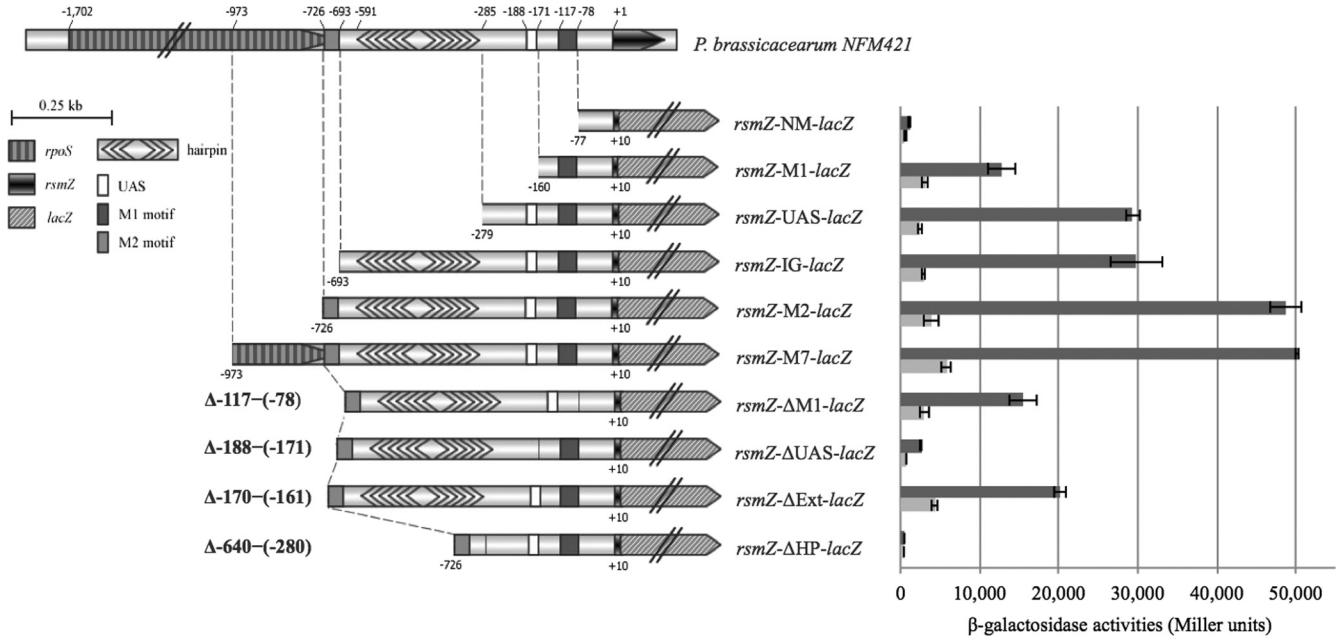

FIG 2 Localization and influence of predicted motifs on *rsmZ* gene expression. Activities of a *lacZ* reporter fused to promoter fragments of *rsmZ* were measured in *P. brassicacearum* wild-type (black bar) and *gacA* mutant (grey bar) strains and are shown as Miller units of β-galactosidase activity (means of three measurements ± standard deviations). Cultures were grown for 24 h in TSB/10 medium at 30°C before the assay.

in *P. aeruginosa* (4), this regulation seems to be GacA dependent in *P. brassicacearum* (Fig. 2).

We do notice that this activating sequence is likely a binding site for a regulator that enhanced *rsmZ* promoter activity, probably by reinforcing GacA interaction with the targeted binding site.

The M2 motif could be referred to as an enhancer since it is a regulatory DNA element that can activate its genomic target over a large distance (>200 bp). Functional enhancer-promoter communication over large distances is facilitated when DNA is supercoiled or bent, thus bringing the enhancer and proximal promoter closer to each other (3). Distortion of the regular DNA conformation may be due to the intrinsic structure or may be induced by DNA binding proteins (24).

Maybe not just a hairpin. The structuring of DNA could be an important factor in *rsmZ* gene expression. Some nucleoid-structuring proteins, like IHF, that facilitate protein-protein interactions between RNA polymerase and upstream activators are involved in *rsmZ* expression (10, 15).

As shown above, the presence of a 308-bp hairpin (with a self-complementary 101-bp stem; 45% GC base pairs) is specific to the

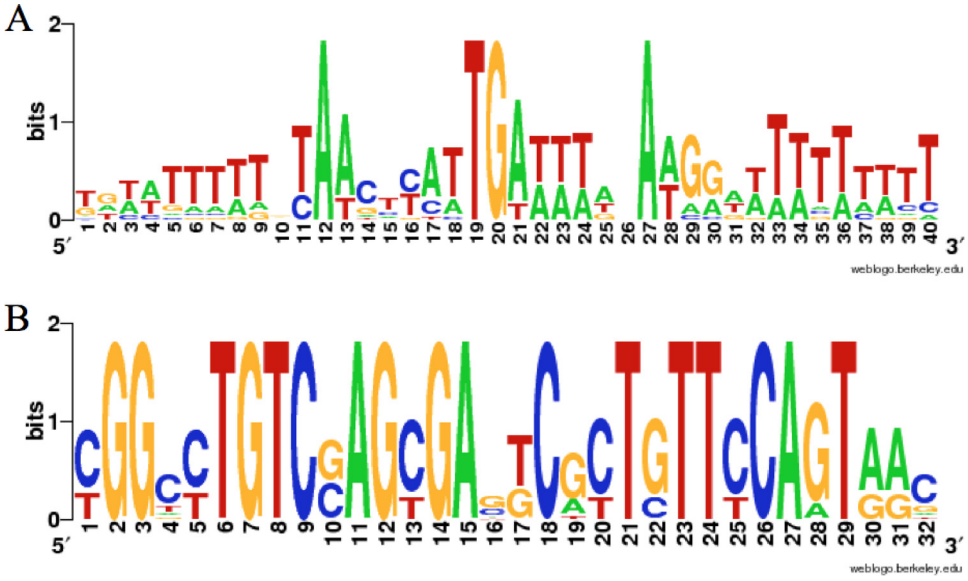

FIG 3 Sequence logo generated via WebLogo software (25) for the M1 motif (A) and the M2 motif (B).

rpoS-rsmZ intergenic region in *P. brassicacearum*. *In vivo* analysis of *rsmZ* gene expression using the *rsmZ*-M7-*lacZ* fusion, in which the hairpin-corresponding sequence (from −640 to −279 bp) has been deleted, revealed a drastic decrease, suggesting the high importance of this hairpin to *rsmZ* activation (Fig. 2). Surprisingly, the absence of the hairpin abolished the activation of *rsmZ* in spite of the presence of the M1 motif and the UAS. This putative hairpin structure may be involved in DNA bending by bringing the distant activator located at the M2 motif into a position where it can directly interact with GacA and/or RNA polymerase. In addition, the absence of the hairpin may favor the binding of a repressor yet to be identified.

Conclusion. The *mutS-rpoS* region has been utilized in gammaproteobacteria to integrate horizontally acquired genetic information for rapid adaptation to their habitat. In contrast to enterobacteria, in *Pseudomonas* this region, where the gene encoding RsmZ is located, is quite stabilized. Our *in vivo* analysis of the *rsmZ*-activating sequences indicates that the conserved palindromic UAS required for GacA-controlled sRNA genes in gammaproteobacteria is essential but not sufficient for full expression of the *rsmZ* gene in *P. brassicacearum* NFM 421; this is due to the fact that a nonnegligible activity is observed with M1 and M2 motifs, which is not completely altered in a *gacA* mutant. Additional subtleties in this regulatory system are suggested by the implication of a putative hairpin, which is likely a key player in *rsmZ* gene activation.

Altogether, our results suggest an intricate multi-input system and emphasize the higher complexity in transcriptional control of the *rsmZ* gene in the *P. brassicacearum* NFM 421 strain.

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